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EVALUATION OF A COMPUTER PROGRAM FOR GC-MS SPECIFIC ION MONITORING

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June 1974

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ABSTRACT

A computer program, "Specific-Ion Mass Spectrometric Detection for Gas Chromatographic Pesticide Analysis" (SIM), developed under an Environmental Protection Agency (EPA) grant, was evaluated at the EPA's Southeast Environmental Research Laboratory. Standard solutions of four chlorinated pesticides were used to compare the SIM program data to those produced by an existing limited-mass data acquisition program. Under similar conditions, similar sensitivities were observed with both programs. Greater sensitivity was obtained with the SIM program when its parameter selection options were fully utilized.

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This report has been assigned to the ENVIRONMENTAL PROTECTION TECHNOLOGY series. This series describes research performed to develop and demonstrate instrumentation, equipment and methodology to repair or prevent environmental degradation from point and non-point sources of pollution. This work provides the new or improved technology required for the control and treatment of pollution sources to meet environmental quality standards.

SECTION I
CONCLUSIONS

With optimum conditions, greater sensitivity was observed with the Specific-Ion Monitoring (SIM) program data acquisition than with System/150 limited-mass data acquisition. The SIM Program is useful for detection of trace quantities of compounds whose fragmentation patterns are known, but ions to be monitored must be carefully selected to avoid background ions. If the compound's significant ions are also background ions, the System/150 program is preferable, because it permits background data subtraction.

SECTION II
INTRODUCTION

Setting and enforcing water quality criteria, determining the fate and effects of water pollutants, and developing optimum control measures require the capability for identifying specific organic pollutants. To be acceptable, the identification technique must be highly specific since thousands of compounds must be considered. Because some organic compounds are toxic to aquatic organisms at concentrations below 10 µg/l, the technique must also be sensitive.

One technique that has become invaluable in pollutant analysis is gas chromatography-mass spectrometry (GC-MS), a method that utilizes a gas chromatograph to separate sample constituents and a mass spectrometer to obtain mass spectra as the sample components elute. A small dedicated computer coupled to the GC-MS greatly decreases data reduction time.

A technique that increases sensitivity for GC-MS detection of particular known compounds is limited-mass data acquisition. The mass spectrometer, instead of being set to scan the entire mass range, is set to monitor only a few significant mass fragments of compounds suspected to be present in a sample. By concentrating on only a few masses, specific ion monitoring is more specific and 10- to 100-fold more sensitive than standard repetitive scanning.

The masses of interest and appropriate GC conditions must be known before specific mass data acquisition can begin. Knowledge of the fragmentation pattern of a compound is not adequate; ions to be monitored must be sufficiently unique to provide definitive information. Some ions are indicative of specific compounds; the presence of the ion and GC retention data together can confirm a tentative identification. Other ions indicate particular classes of compounds.

Ions to be monitored must be carefully chosen to avoid background ions such as those produced by column bleed and pump oil and to be specific for the suspected compound. With the dilute samples that require this special technique, even a relatively insignificant background fragment can produce a stronger signal than the sample fragment being monitored. Slight variations in background intensity may then produce plotter pen displacements as large or larger than those caused by sample ions.

The chosen ions must be sufficiently intense to provide reasonable sensitivity. If more than one fragment is monitored for a compound, the ratio of intensities provides additional confirmatory information. Quantitation can be achieved by comparing sample peak areas with those of standards.

The repetitive-scanning standard data acquisition program of System/150 could be used for limited-mass data acquisition if the operator entered one or a few masses for each mass range to be scanned and set a relatively long integration time for each. However, some desirable features were not available with the standard repetitive scanning program.

With the standard program, the masses had to be monitored in ascending order. Program parameters could not be changed without halting data acquisition and initiating another data file. The standard program monitored the same set of ions during the entire run, although the ions of interest changed as different sample components eluted. Therefore, the standard program had less sensitivity than could be obtained with a program that permitted masses to be changed.

A program more suited to the specialized technique was needed and was developed at Battelle Columbus Laboratories

under Environmental Protection Agency Grant #R-800909. This report summarizes the Southeast Environmental Research Laboratory's evaluation of this program, Specific-Ion Monitoring, and compares its performance to that of the standard repetitive-scanning program.

SECTION III
EXPERIMENTAL

INSTRUMENTATION

Mass spectral data were obtained with a Finnigan 1015 S/L electron-impact quadrupole mass spectrometer operated with the following conditions: 70eV electron energy, 10^{-6} sensitivity range, and 450 μa ionizing current. A modified Varian 1400 gas chromatograph was interfaced to the mass spectrometer with a Gohlke glass jet separator. The chromatograph was fitted with a 6 ft x 0.25 in (O.D.) glass column packed with 3% Silar on 80-100 mesh Gas Chrom Q. The column was operated at 240° C isothermal with helium flowing at approximately 18 ml/min. Data acquisition and processing were controlled by System Industries System/150, which interfaced the mass spectrometer with a 4K Digital Equipment Corporation PDP 8/e computer.

SAMPLE PREPARATION

A stock solution containing four chlorinated pesticides (o,p'-DDD, p,p'-DDE, p,p'-DDT, and dieldrin) was prepared by dissolving 10 mg of each pesticide in 1ℓ of hexane. Aliquots of the stock solution were diluted to give solutions with the following concentrations: 5 ng/μℓ, 1 ng/μℓ, 0.5 ng/μℓ, and 0.2 ng/μℓ.

SIM PROGRAM

The SIM program[1] was written for PDP 8/e computers with the KE8-E extended arithmetic element (EAE). Subroutines were added for PDP 8/e computers without an EAE. Data for evaluation were acquired with the non-EAE version.

1. Data Acquisition

Data acquisition conditions, or parameters, are specified by teletype communication between analyst and computer (Figure 1). From one to eight m/e values are entered as a set of masses to be monitored. An integration time (in milliseconds) can be specified for each mass, or all masses can be monitored with the same integration time. The "NO. POINTS" prompt requests the analyst to specify the number of times a set of masses is to be monitored before the computer is to add the acquired data and store the sum.

```
SYSTEM-15Ø IS ON SELECT MODE:  USER
LOAD FILE:  SIMSCN
CALIBRATION FILE: CALI
MASS(ES): 246;248
INTEGRA. TIME:45Ø;45Ø
ANOTHER SET?Y
MASS(ES): 235;237
INTEGRA. TIME:45Ø
ANOTHER SET?Y
MASS(ES): 263
INTEGRA. TIME: 75Ø
NO. POINTS: 5
RUN TIME: 3Ø
TITLE:PESTICIDE MIXTURE
SCALE FACTOR: 8
DATA FILE NAME: MIX
DATA
```

Figure 1. SIM program initiation dialogue

After program initiation, integration times and the number of points can be changed; data acquisition must be halted only long enough to enter new parameters with appropriate teletype characters. Three sets of masses, which are designated during program initiation, may be monitored in any order with teletype prompts.

2. Data Output

The m/e value first specified to be monitored is plotted as data are acquired; real-time plot amplitude is controlled by the scale factor entered via teletype during program initia-

tion. After data acquisition is halted, data for all masses are plotted either overlaid or separately. In either case, plots are normalized to the most intense signal. Since the program does not include a background subtraction routine, the most intense signal may be due to ions from column bleed, pump oil, or other spectrometer contaminants. The Savitsky-Golay least-squares smoothing routine[2] is used to plot data.

SECTION IV
DISCUSSION

The SIM program and standard System/150 specific-mass detection were compared using data obtained for solutions containing a mixture of four chlorinated pesticides. After satisfactory chromatographic conditions were established for the mixture of four chlorinated pesticides (o,p'-DDD, p,p'-DDE, p,p'-DDT, and dieldrin), a complete mass spectrum was obtained for each compound. To determine which masses should not be used for SIM monitoring, background and column-bleed spectra were recorded with the same instrumental conditions that were used for the pesticide spectra.

Both p,p'-DDT and o,p'-DDD had base peaks at m/e 235 and 67% fragments at m/e 237. Background ions with these m/e values were insignificant. The third most intense peak in both spectra was m/e 165; a significant background contribution precluded monitoring this ion. Accordingly m/e 235 and 237 were selected for both compounds, which could be distinguished from each other by GC retention times.

For p,p'-DDE, a unique base peak of m/e 246 along with a strong m/e 248 (65% of base peak) provided excellent specificity and sensitivity. The absence of background interference and the intensity of these peaks made them ideal ions to monitor.

No ideal ion was found to monitor dieldrin. Background ions interfered with the base peak at m/e 79 and the three other strong peaks (m/e 81, 35%; 82, 30%; and 77, 25%). In SIM trials runs, fluctuations in background ion intensity at these masses produced "peaks" as strong as those from 1 ng of dieldrin. Mass 263, although only 5% of base peak intensity, was used to monitor dieldrin, because background ions at mass 263 were insignificant.

After the ions to be monitored were chosen, several runs were made with the SIM program to determine the best program parameters. (The relationship between integration times and number of points affects the apparent GC resolution and peak sharpness.) Best results were obtained when only masses 235, 246, and 263 were monitored with 250 msec integration time for each. Five points were added before the sum of intensities was stored. Data were output as superimposed plots with each mass plotted separately (Figure 2). All data points in a particular data file were normalized with the smallest stored number set equal to zero and the largest stored number set equal to 100% relative intensity.

With the spectrometer conditions current when these data were acquired, 1 μℓ of the 1-ng/μℓ solution was needed to observe all four pesticides, but DDT and dieldrin were often barely discernible. With a 1-μℓ injection of the 0.2-ng/μℓ solution (Figure 3), DDE (m/e 246) and (m/e 235) were easily observed, but dieldrin and DDT were not discernible. Monitoring only m/e 246 or 235 would increase the apparent sensitivity. However, no attempt was made to determine a detection limit for each compound. The limit varies with instrumental conditions, such as tuning, electron multiplier condition, spectrometer contamination, quadrupole cleanliness, separator condition, and electronic stability.

Since data are output only as normalized plots, quantitative information can be obtained only be comparing relative peak heights or relative peak areas. To determine SIM peak reproducibility, six consecutive 1-μℓ injections of the 1-ng/μℓ solution were monitored. All instrumental conditions were maintained as constant as possible, and the mass

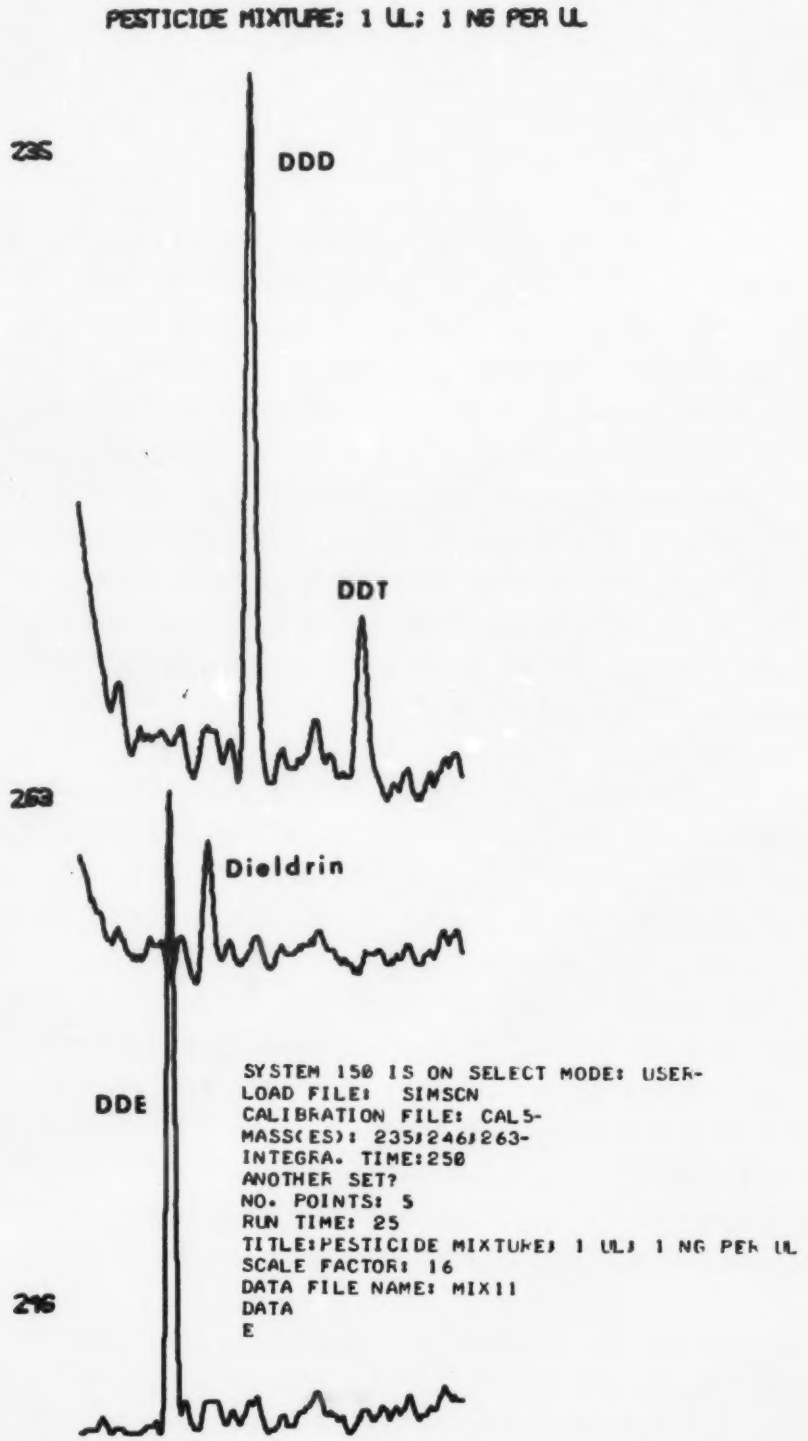

Figure 2. Plotter output of SIM data obtained from 1 ng each of four pesticides

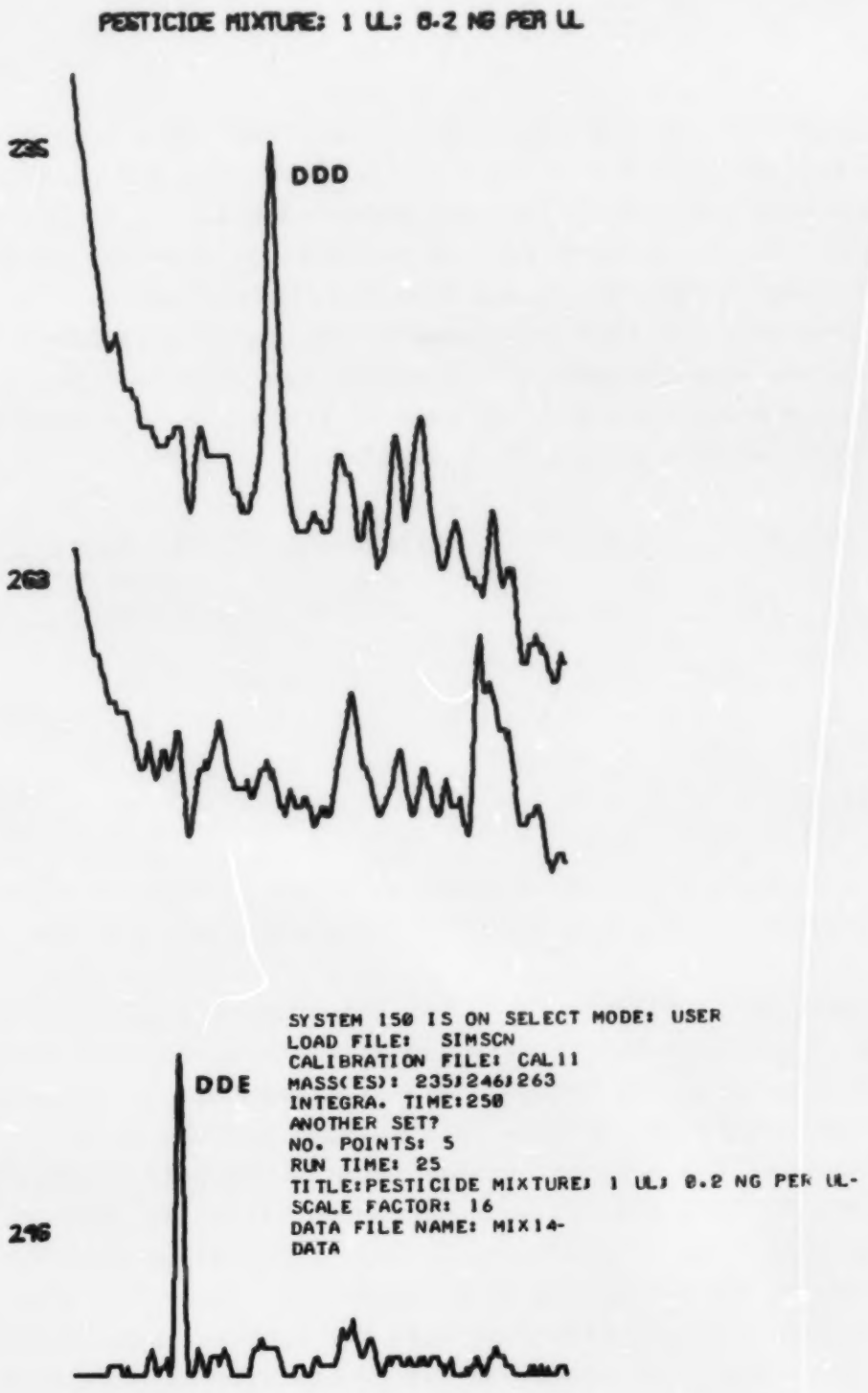

Figure 3. Plotter output of SIM data obtained from 0.2 ng each of four pesticides

spectrometer was mass calibrated before each data acquisition. From the specific ion chromatograms, peak-area and peak-height ratios were calculated for each compound relative to o,p'-DDD, which produced the most intense response of the four compounds. The results (Table 1) showed that peak-height ratios were more reproducible than peak-area ratios calculated from planimeter measurements. This result was also reported by Holland and co-workers.[3] Because of the noise, the greatest source of error was construction of peak baselines.

Table 1. COEFFICIENTS OF VARIATION FOR SIM DATA

	Peak heights	Peak areas[a]
p,p'-DDT / o,p'-DDD	18%	24%
p,p'-DDE / o,p'-DDD	11%	12%
Dieldrin / o,p'-DDD	8%	8%

[a]Measurements corrected for error in planimeter readings

Data were also collected for four consecutive 1-μℓ injections of the 1-ng/μℓ pesticide solution with the standard System/150 data acquisition method. All instrumental conditions were maintained as constant as possible, and the mass spectrometer was mass calibrated before each data acquisition. Masses 235, 246, and 263 were monitored with 1000 msec integration time for each mass. This integration time was arrived at experimentally as a compromise. A longer time theoretically would have increased the apparent sensitivity but also would have increased the probability of missing a large portion of some GC peaks. Data were output as total ion current plots with an overlaid limited-mass ion current

plot of each significant ion (Figure 4). Three pesticides, DDE, DDD, and DDT were readily apparent in the computer-reconstructed chromatogram, but dieldrin was not detected in either the reconstructed chromatogram or the m/e 263 limited-mass plot. DDT appeared as a small, broad peak and would probably not have been apparent with GC retention-time information.

To compare reproducibility of SIM and System/150 data, coefficients of variation were calculated for both peak heights and peak areas (Table 2).

Table 2. COEFFICIENTS OF VARIATION FOR SIM AND SYSTEM/150 DATA

	Peak Heights		Peak Areas	
	SIM	System/150	SIM	System/150
DDD	9%	11%	12%	17%
DDE	2%	7%	15%	8%
DDT	13%	22%	8%	7%
Dieldrin	13%	--	12%	--

Whereas System/150 requires that masses be monitored in ascending order, the SIM program permits masses to be monitored in any order. System/150 monitoring of masses 235, 237, 246, and 248 for a mixture of DDD, DDE, and DDT is hampered by the ascending mass order requirement. With the relatively long integration times required, too much time would be spent on masses 235 and 237 (indicative of DDD and DDT) while the DDE peak (masses 246 and 248) eluted. Likewise, much of a sharp DDD peak could elute and be missed while masses 235 and 237 were monitored. The SIM program permits alternating the masses indicative of DDE with masses indicative of DDD and DDT, and masses can be monitored in the order: 235, 246, 237, and 248.

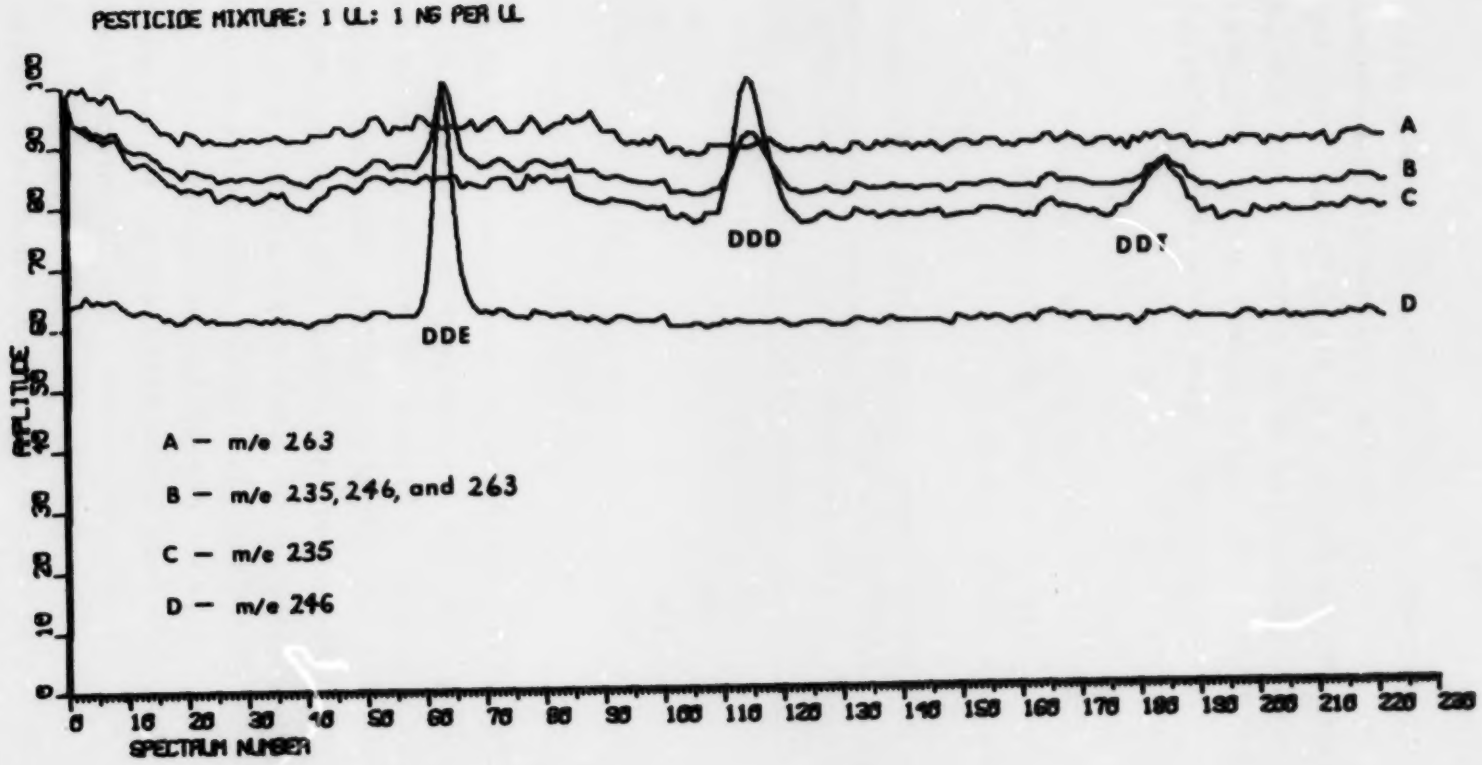

Figure 4. System/150 data obtained from a solution containing 1 ng each of four pesticides

The SIM program permits data acquisition parameters to be changed with minimal interruption of data acquisition and without initiating a new data file, which is required with System/150. Increased sensitivity was obtained by using the SIM program's three sets of masses to devote all scan time to the mass of interest for each GC peak (Figure 5). During program intiation, the appropriate mass was designated for each set. The first set, m/e 246, was monitored until the first peak (DDE) eluted. The second set, m/e 263, was monitored until the second peak (dieldrin) was observed, and the third set, m/e 235, was monitored for DDD and DDT, the third and fourth peaks, respectively. For each set change, data acquisition was halted only long enough to enter two characters via teletype.

A significant disadvantage of the SIM program is the inability to eliminate background data. SIM data plots are normalized to the most intense signal, which might be composed mainly of background signals with only a small contribution from sample. Since System/150 data are stored as discrete spectra, background data can be subtracted and the appropriate spectrum plotted to show relative intensities of monitored ions. For example, both m/e 235 and 237 must be present to confirm the presence of DDT, and their relative intensities are significant information. An approximation of sample concentration can be obtained with System/150 data by comparison of the absolute number of counts for the sample peaks (after background subtraction) with those for a standard analyzed with the same conditions. However, since SIM data are output as normalized plots, concentration estimates require internal standards.

The SIM program version used to collect these data contained some annoying bugs. An operator typographical error during program parameter changes (such as typing a character not

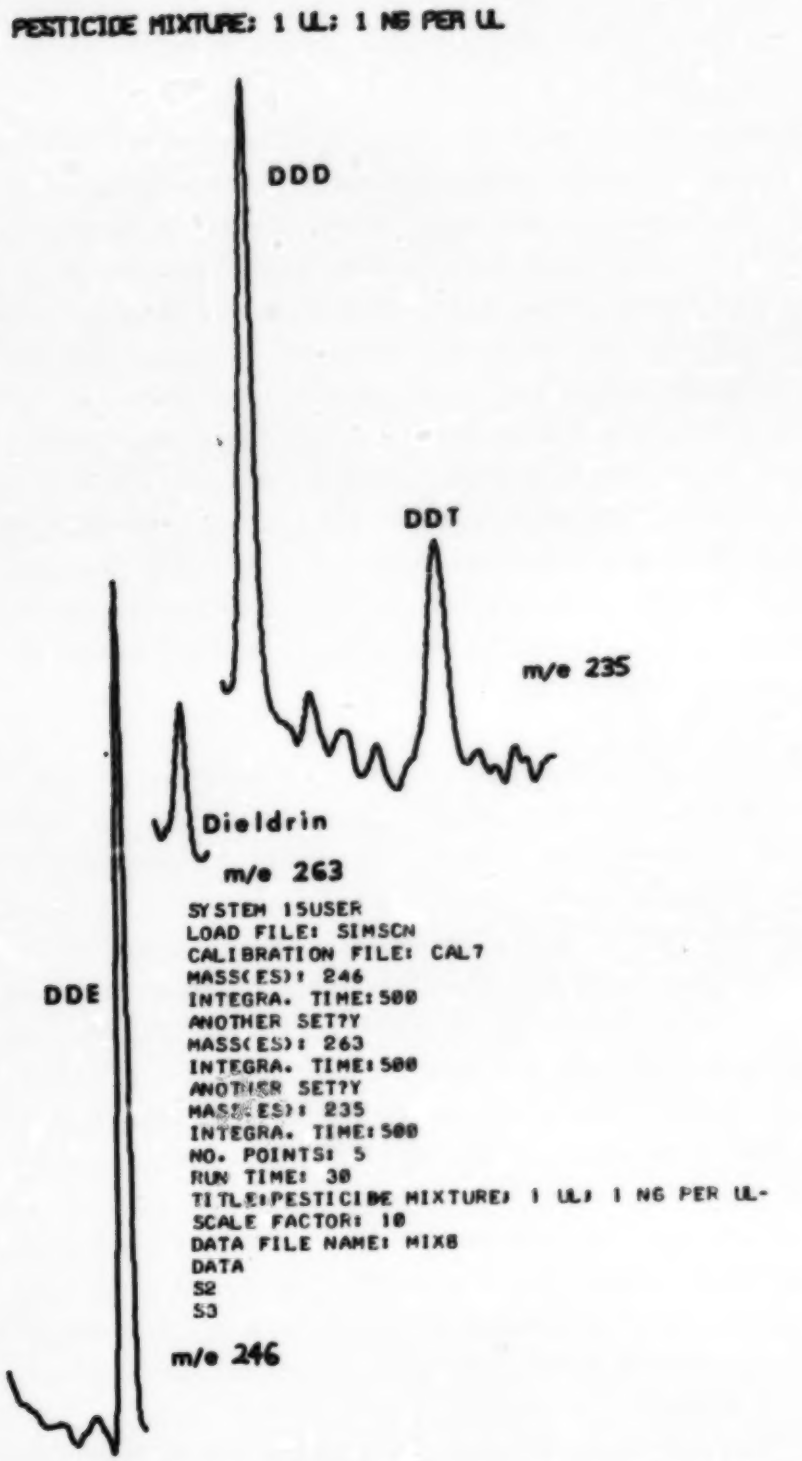

Figure 5. SIM data obtained from 1 ng each of four pesticides when three mass sets were used

recognized as a parameter designator) halted data acquisition without the proper termination sequence. The data file was therefore inaccessible. Manual termination of data acquisition had to be done cautiously. When the teletype did not echo the termination character "E" before the carriage return was activated, the data file was not terminated properly and was therefore inaccessible.

Interferences with SIM data acquisition were observed after a Tektronix 4012 cathode ray tube (CRT) was integrated into the GC-MS-Computer system. Local-mode graphics CRT operation produced major plotter pen displacements on both real-time and final output. No evidence was found that local-mode CRT operation interfered with System/150 data acquisition.

SECTION V

REFERENCES

1. Neher, M. B., and J. R. Hoyland. Specific-Ion Mass Spectrometric Detection for Gas Chromatographic Pesticide Analysis. Battelle Columbus Laboratories, Columbus, Ohio. Environmental Protection Agency Publication Number EPA-660/2-74-004. January 1974.

2. Savitsky, A., and M. J. E. Golay. Smoothing and Differentiation of Data by Simplified Least Squares Procedure. Anal. Chem. 8: 1627-1638, 1964.

3. Holland, J. F., C. C. Sweeley, R. E. Thrush, R. E. Teets, and M. A. Bieber. On-Line Computer Controlled Multiple Ion Detection in Combined Gas Chromatography-Mass Spectrometry. Anal. Chem. 45: 308-314, February 1973.

SECTION VI

APPENDIX

SIM Program Dialogue

MASS(ES): From one to eight masses are designated; the first mass specified is displayed on the plotter during data acquisition.

INTEGRATION TIME: Response specifies the time (1 to 4096 milliseconds) each mass is to be monitored. Integration times are paired with masses previously entered; the first integration time is used for the first mass designated etc. When more masses than integration times are entered, the last time is used for masses that have no corresponding time. If all masses are to be scanned for the same period, only one time value must be entered.

ANOTHER SET?: A positive response allows up to eight masses to be entered to form a new mass set (maximum of three sets).

NUMBER OF POINTS: Response specifies how many times the mass set is scanned before values for each mass are summed and stored.

RUN TIME: After number of minutes specified, data acquisition will be automatically halted.

SCALE FACTOR: Amplification factor controls real-time plot amplitude.

The SIM Program permits some parameters to be changed with operator-computer communication via teletype during data acquisition. Permissible parameter designators are

- E - terminates data acquisition before expiration of time entered during program initiation
- F - followed by number - changes real-time amplification factor
- N - followed by number - changes the number of times each mass is scanned before values are summed and stored

S - followed by number 1, 2, or 3 - begins data acquisition for a different mass set which was entered during program initiation

T - followed by number(s) - changes integration time for the masses being monitored to the time (milliseconds) specified with new number